Effect of standardized aqueous extract of Withania somnifera on tests of cognitive and psychomotor performance in healthy human participants

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Abstract

Background: Withania somnifera is an herbal medicine that has been known to possess memory-enhancing properties. The current study involved an assessment of cognitive and psychomotor effects of Withania somnifera extract in healthy human participants. Materials and Methods: In this prospective, double-blind, multi-dose, placebo-controlled, crossover study, 20 healthy male participants were randomized to receive 250 mg two capsules twice daily of an encapsulated dried aqueous extract of roots and leaves of Withania somnifera or a matching placebo for a period of 14 days. Cognitive and psychomotor performance was assessed pre-dose (day 1) and at 3 hrs post-dose on day 15 using a battery of computerized psychometric tests. After a washout period of 14 days, the subjects crossed-over to receive the other treatment for a further period of 14 days as per prior randomization schedule. Same battery of test procedures were performed to assess cognitive and psychomotor performance. Results: Significant improvements were observed in reaction times with simple reaction, choice discrimination, digit symbol substitution, digit vigilance, and card sorting tests with Withania somnifera extract compared to placebo. However, no effect can be seen with the finger tapping test. Conclusion: These results suggest that Withania somnifera extract can improve cognitive and psychomotor performance and may, therefore, be a valuable adjunct in the treatment of diseases associated with cognitive impairment.

Materials and Methods

Study medication
Each capsule contains either an inert placebo or 250 mg of highly standardized dried aqueous extract of the roots and leaves of Withania somnifera [11] (SENSORIL® , Natreon Inc, India)
In this test, the participant was asked to sort a set of 52 cards based on the different colors and shape using a digital pen. Results are presented in terms of the average time taken to sort the cards.

**Card sorting test**

Screen at the rate of 105 digits/min. The subject has to press the "Enter Button" on the response box as quickly as possible every time the digit in the series matched the target digit. The target digit was randomly selected and constantly displayed to the right of the monitor screen. On click of start button, a series of digits will appear one at a time on the center of the screen. The subject has to press the corresponding digit as quickly as possible on the response box when the target symbol appears. The total duration of the test is 90 sec. Results are presented in terms of average reaction time, total attempts, correct attempts, and wrong attempts. This test assesses attention, response speed, central integration, and visuo-motor coordination.

**Digit symbol substitution test**

The upper panel of the screen will display 1-9 digits with their corresponding target symbol placed over each digit. The subject has to carefully concentrate and remember the corresponding digit for these symbols. On click of start button, the symbols that are shown in the panel will appear randomly one after another on the center of the screen. The subject has to press the 'BOY' symbol button with right index finger and 'GIRL' symbol button with left index finger on the response box as quickly as possible corresponding to the picture. Each time, the picture will remain on the screen for 1 sec, and there will be a time gap of 1.5-2.5 sec between the appearances of subsequent BOY pictures. Results are presented in terms of average reaction time, correct attempts, and wrong attempts. This test assesses attention and sensory-motor performance of brain.

**Choice discrimination test**

In this test, the upper panel of the screen will display 1-9 digits with their corresponding target symbol placed over each digit. On click of start button, the symbols that are shown in the panel will appear randomly one after another on the center of the screen. The subject has to press the corresponding digit as quickly as possible on the response box when the target symbol appears. The total duration of the test is 90 sec. Results are presented in terms of average reaction time, total attempts, correct attempts, and wrong attempts. This test assesses attention, response speed, central integration, and visuo-motor coordination.

**Digit vigilance task**

A target digit was randomly selected and constantly displayed to the right of the monitor screen. On click of start button, a series of digits will appear one at a time on the center of the screen at the rate of 105 digits/min. The subject has to press the "Enter Button" on the response box as quickly as possible every time the digit in the series matched the target digit. The task lasts for 60 sec, and there will be 45 stimulus-target matches. The results are presented in terms of average reaction time (milliseconds), total attempts, correct attempts, and wrong attempts. The test assesses attention, response speed, central integration, and visuo-motor coordination.

**Card sorting test**

The tests used in the study are sensitive and can detect drug-induced changes in psychomotor performance in healthy subjects. The entire session of tests presented took approximately 20 minutes, and in each session, tests were administered in the following order. Each test was done thrice, and an average of three readings was taken.

**Finger tapping test**

The duration of the test is 10 sec, during which the subject has to continuously tap on the "Enter Button" on the response box in quick succession. The results were presented in terms of average reaction time in milliseconds as well as total number of clicks attempted. The test provides information on motor system performance.

**Simple reaction test**

The duration of the test is 60 sec. In this test, on the click of a start button, the test time will begin and a picture of a boy will appear on the center of the screen for 20 times. The subject has to press the 'BOY' symbol button on response box as quickly as possible every time the 'BOY' picture appears on the monitor. Each time, the BOY picture will remain on the screen for 1 sec, and there will be a time gap of 1.5-2.5 sec between the appearances of subsequent BOY pictures. Results are presented in terms of average reaction time, correct attempts, and wrong attempts. This test assesses attention and sensory-motor performance of brain.

**Choice discrimination test**

The duration of the test is 60 sec. In this test, on the click of a start button, the test time will begin and a picture of a boy or girl will appear randomly for 10 times each on the center of the screen. The subject has to press the 'BOY' symbol button with right index finger and 'GIRL' symbol button with left index finger on the response box as quickly as possible corresponding to the picture. Each time, the picture will remain on the screen for 1 sec, and there will be a time gap of 1.5-2.5 sec between the appearances of the subsequent pictures. Results are presented in terms of average reaction time, correct attempts, and wrong attempts. This test assesses attention and sensory-motor performance of the brain and estimates the psychomotor response speed.

**Digit symbol substitution test**

In this test, the upper panel of the screen will display 1-9 digits with their corresponding target symbol placed over each digit. The subject has to carefully concentrate and remember the corresponding digit for these symbols. On click of start button, the symbols that are shown in the panel will appear randomly one after another on the center of the screen. The subject has to press the corresponding digit as quickly as possible on the response box when the target symbol appears. The total duration of the test is 90 sec. Results are presented in terms of average reaction time, total attempts, correct attempts, and wrong attempts. This test assesses attention, response speed, central integration, and visuo-motor coordination.

**Digit vigilance task**

A target digit was randomly selected and constantly displayed to the right of the monitor screen. On click of start button, a series of digits will appear one at a time on the center of the screen at the rate of 105 digits/sec. The subject has to press the "Enter Button" on the response box as quickly as possible every time the digit in the series matched the target digit. The task lasts for 60 sec, and there will be 45 stimulus-target matches. The results are presented in terms of average reaction time (milliseconds), total attempts, correct attempts, and wrong attempts. The test assesses attention, response speed, central integration, and visuo-motor coordination.
In this test, the participant was asked to sort a set of 52 cards based on the different colors and shape using a digital pen. Results are presented in terms of the average time taken to complete the sorting and the number of correct and wrong cards. This test assesses sensory, motor, central integrative and executive functions.

Safety assessments
Physical examination, systemic examination, vital signs, hematological and laboratory tests like hemoglobin, complete blood picture, renal function tests, liver function tests, lipid profile, and complete urine examination including ECG were performed at screening visit, baseline, and end of treatment during each cross-over period.

Statistical analysis
The statistical analysis was carried using Graphpad prism software, Version 4 (Graphpad software Inc. Sandiego, California, USA). All the data were presented as Mean ± SD. Within subject and pair-wise comparisons between the two treatments (Withania somnifera extract Vs Placebo) were tested for statistical significance using one way ANOVA and paired t-test. Statistical significance was at P < 0.05.

Sample size calculation
Assuming a 15% difference between two treatments, sample size of 20 subjects was calculated based on two-sided differences with the type I error α being 0.05 and type II error β at 80%. Taking into account study design and total number of times subject visiting the study center and expected dropout of 30%, a total of 26 subjects were enrolled into the study.

Results

Total 26 subjects were screened, out of which four subjects were excluded because of abnormal laboratory investigations and two subjects did not report to randomization visit.

The mean age of the 20 subjects was 24.90 ± 4.18 yrs, with a mean BMI of 22.38 ± 1.15 kg/m 2. There were no significant differences in baseline characteristics between the two groups.

The results of the outcome measures are depicted in [Table 1]. As seen from the table, Withania somnifera extract significantly decreased reaction time (RT) in SRT, CDT, DSST, DVT, and CST compared to that of baseline and placebo. However, no significant effect can be seen with FTT.(Table 1)

Compared to placebo, the mean percentage change in reaction time for the CDT, DSST, and DVT decreased significantly. However, no significant change can be seen with FTT, SRT, and CST [Figure 2] and [Figure 3].

All subjects completed both treatments, and no participant discontinued the study. Compliance to study medication was good, and all the subjects in the Withania somnifera extract group and in the placebo group took more than 90% of the study drugs. Safety laboratory parameters were within normal range, and none of the treatments affected the safety lab parameters.

Discussion

The present study demonstrated the favorable effects of Withania somnifera extract on cognitive and psychomotor performance in healthy participants. Psychometric tests like FTT, CDT, DSST, DVT, and CST were performed using computerized psychometric test system, which have gained popularity in recent times in clinical studies of psychiatric disorders and to evaluate the effects of psychotropic drugs. [13] Psychometric tests are used as biological markers in early phase 1 trials to establish preliminary objective pharmacodynamic data. [1] In the present study, the psychomotor performance tests used are well-established with sensitivity to the depressant and stimulant effects for a wide range of psychotropic drugs.

Withania somnifera is a known brain tonic in Ayurveda postulated to act in the human body by modulating the neuro-endocrino-immune systems and have been found to be a rich source of antioxidants.

In the present study, we have observed that Withania somnifera extract decreased the reaction time significantly in SRT, CDT, DSST, DVT, and CST following 14 days treatment (1000 mg/day) compared to placebo, indicating its positive effect on psychomotor function. Similarly, there was significant reduction in mean percent reaction time by 6% in SRT, 7% in DSST, and 3% in DVT compared to placebo.

The decrease in the reaction time could be interpreted as a central effect, since Withania somnifera helps to maintain homeostasis and psychomotor abilities in times of stress. Clinical trials have shown that Withania somnifera can alleviate a reactive type of depression without causing sedation. Instead, it optimizes mental and psychomotor performance by easing the mental stress bundle. [8] Our results are in par with the above data where we have not witnessed any sedative effect in the subjects during treatment with Withania somnifera extract. The mechanism by which Withania somnifera exerted beneficial effects is presently not clear; however, animal studies have shown that Withania somnifera is capable of improving memory and enhancing cognitive function by modulation of cholinergic neurotransmission. As per the literature, leaf and fruit extracts of Withania somnifera consisting of mixtures of sitoindosides VII-X and withaferin-A produces an increase in the cortical muscarinic acetylcholine capacity, which may partly explain cognition-enhancing effect demonstrated in animal and human models. Schliebs et al. [10] studied the effects of sitoindosides VII-X and withaferin isolated from aqueous methanol extracts from the roots of Withania somnifera on brain cholinergic, glutamatergic, and GABAergic receptors in male Wistar rats. The activity of AChE levels is enhanced in lateral septum and globus pallidus, whereas it is decreased in vertical diagonal band. Similarly, the activity of M1 cholinergic receptor binding is enhanced in lateral septum and frontal cortex, whereas M2 receptor binding sites increased in frontal and parietal cortex regions. The data suggests that the compounds - sitoindosides VII-X and withaferin preferentially affect events in the cortical cholinergic-signal transduction cascade. The drug-induced increase in cortical muscarinic acetylcholine receptor capacity might partly explain the cognition-enhancing and memory-improving effects of Withania somnifera extract in animals and in humans.

Similarly, Dhuley et al. [14] tested Withania somnifera, comprising sitoindosides VII-X and Withaferin-A, to prove their effect on improving short-term memory in mice. The test was done using electric shock therapy, treatment with scopolamine (an anti-cholinergic agent that produces deficits in learning and memory retention) as well as testing mice that had never been on the electric grid before. The mice treated with Withania somnifera (200 mg/kg of body weight) produced a significant decrease in the amount of errors while trying to reach the shock-free zone after 1 hr of training. The effect of Withania somnifera on the scopolamine-treated mice reversed the deficits in retrieval and acquisition. This study showed an effectiveness of Withania somnifera in improving short-term memory in both naive and amnesiac mice.

Though there is not much data on the effects of Withania somnifera on psychomotor performance in healthy participants, research by Karnick CR [15] in a randomized, double-blind study reported that Withania somnifera significantly improved integrated sensorimotor function, audition reaction time, and mental arithmetic ability compared to Panax ginseng and placebo in...
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### Conclusion

The measurement of cognitive and psychomotor performance is highly important in evaluating and predicting treatment outcomes in healthy subjects as well as in patients. In the present study, *Withania somnifera* extract has shown to improve the cognitive and psychomotor performance in healthy human participants. However, multicentric long-term clinical studies in patients are required to confirm its therapeutic efficacy in disease states associated with impaired cognition and psychomotor function.

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